Detection of anti-tissue transglutaminase antibodies in autoimmune thyroid disease

Seo-Jin Park

Department of Medicine

The Graduate School, Yonsei University

Detection of anti-tissue transglutaminase antibodies in autoimmune thyroid disease

Directed by Professor Kyung Soon Song

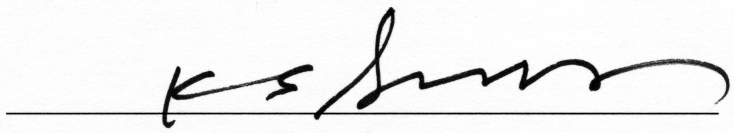
The Master's Thesis
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the
degree of Master of Medicine

Seo-Jin Park

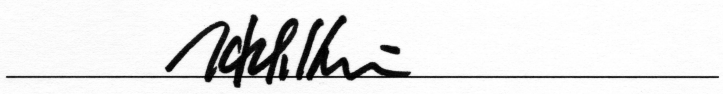
June 2006

This certifies that the Master's Thesis of

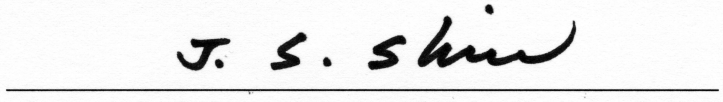
Seo-Jin Park is approved.



Thesis Supervisor: Kyung Soon Song



Thesis Committee Member: Hyon Suk Kim



Thesis Committee Member: Jeon-Soo Shin

The Graduate School

Yonsei University

June 2006

ACKNOWLEDGEMENTS

I am most grateful to my thesis supervisor, Professor Kyung Soon Song who has always been a very supportive and steady mentor, patiently guiding me through my resident training. Her passion for academic pursuit supported by an immense amount of knowledge in various areas of medicine has been, and always will be an inspiration to me, and to those who have had the fortune to do collaborative work.

I owe my deep appreciation to Professor Hyon Suk Kim, who has shown constant interest and provided guidance throughout my experiment as well as my resident training. I would especially like to thank her for allowing me to "invade" the immunology laboratory on weekends and evenings for my experiment.

I would like to express my gratitude to Associate Professor Jeon-Soo Shin, who offered due criticism and advice when needed with his exceptional analytical thinking, for restructuring my thesis in the preliminary stages of my research. I have been motivated by the genuine interest he has shown in my work.

I am also very lucky to have the support of Professor Jeong-Ho Kim, who provided the samples for the normal group by sharing his research material. I would never have gotten here without the help of my brilliant colleague and friend Youn Hee Park and many thanks to Juwon Kim for her meticulous collection of the samples I desperately needed.

Last but not least, I could never have finished my thesis on time without the support of the dearest and most precious people I have come to know, my family. I have survived my insecurities through endless encouragement from the big sis, Seo-Hyun and her husband, Il-Hyun. I thank Chang-Seo for parting with his newly acquired 1G USB memory drive, which has helped me throughout my research. Above all, I am greatly indebted to my parents for their deep love and support for "the second child," who was quite a handful in her teenage years. Thank you and I love you.

June 2006

Seo-Jin Park

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Detection of anti-tissue transglutaminase antibodies in autoimmune thyroid disease

Seo-Jin Park

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Kyung Soon Song)

Introduction: Tissue transglutaminase has been revealed as a diagnostic marker for celiac disease and IgG antibodies against tissue transglutaminase (tTG) has been implicated in other autoimmune disorders. The relatively common association between autoimmune thyroid disease (AITD) and celiac disease has prompted us to perform an antibody "screening" in patients with autoimmune thyroid disease.

Materials and Methods: Sera from 127 patients with various thyroid disorders, including Graves' disease (GD), Hashimoto's thyroiditis (HT), hyperthyroidism, hypothyroidism, thyroid nodule, and thyroid cancer and 28 healthy individuals were tested for IgA and IgG anti-tTG antibodies using an ELISA kit (Immuno-Biological Laboratories, Germany).

Results: The median concentrations of IgA and IgG anti-tTG antibodies were significantly higher in the patient group compared to the normal group (IgA: 1.882 vs 1.820 U/mL, p=0.0112; IgG: 3.442 vs 3.225 U/mL, p=0.0239)

whereas no significant difference was seen in the positive rates (IgA: 19.7% vs 7.1%, IgG: 15.0% vs 7.1%). IgA anti-tTG antibody was positive in 8 (3 GD + 5 HT) out of 63 AITD patients. Of the 63 AITD patients, 7 IgA negative patients (3 GD + 4 HT) and 1 IgA positive patient (HT) were positive for IgG anti-tTG antibodies. However, the positive rates of anti-tTG antibodies in AITD patients did not differ from the normal group (IgA and IgG: 12.7% vs 7.1%). There was no significant difference in anti-tTG Ab positive rates in association with anti-thyroid antibodies among GD (n=28), HT (n=35), thyroid cancer (n=32), and other thyroid disease groups (n=32).

Conclusions: Above findings suggest a lack of association between anti-tTG antibodies and specific autoimmune thyroid diseases. Nevertheless, the clinical significance of higher anti-tTG antibody titers in patients with thyroid cancer and other thyroid disorders should be further investigated.

Key words: anti-tissue transglutaminase antibody, autoimmune thyroid disease, anti-thyroid peroxidase antibody

Detection of anti-tissue transglutaminase antibodies in autoimmune thyroid disease

Seo-Jin Park

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Kyung Soon Song)

I. INTRODUCTION

Patients with one autoimmune endocrine disorder have an increased risk of developing another autoimmune disease. The relationship between autoimmune thyroid disease (AITD) and celiac disease (CD) is relatively common, especially since they are all considered to be autoimmune in its pathogenesis.[1-2] Patients with AITD usually have a combination of different thyroid autoantibodies; nearly all patients with Hashimoto's thyroiditis (HT) have anti-thyroid peroxidase (TPO) antibodies (also known as anti-microsomal antibodies) and anti-thyroglobulin antibodies whereas they are seen in 50% to 70% of Graves' disease (GD) patients. However, in cases without symptoms or with low antibody titers, the mere presence of autoantibodies indicates thyroid autoimmunity, not AITD. There is some hypothesis that gluten may be the primary antigen which initiates the formation of antithyroid antibodies as well as antibodies directed against other endocrine glands.

Celiac disease is a chronic disease of the small bowel caused by gluten intolerance in genetically predisposed people with several features of autoimmunity such as HLA linkage, associated autoimmune disease (type 1 DM, AITD, systemic lupus erythematosus (SLE), Sjogren's syndrome), intestinal lymphomononuclear infiltration, presence of specific autoantibodies, and the existence of defined autoantigens.[1,3-4] The determination of serum levels of IgA and IgG anti-gliadin antibodies, IgA anti-endomysial antibodies, and IgA anti-tissue transglutaminase antibodies (anti-tTG Ab), plays an important role in the screening and diagnosis of celiac disease.[5]

Since the discovery of the enzyme transglutaminase (TG) in 1957 by Clarke et al., proteins showing TG activity have been described in microorganisms, plants, invertebrates, amphibians, fish and birds.[6] The TG family includes intracellular (the ubiquitous tissue TG and three different isoenzymes specifically expressed in the epidermis) and extracellular enzymes (factor XIIIa and prostate TG). This enzyme is synthesized by a broad spectrum of all cell types and is widely distributed in human organs and functions by catalyzing the post-translational modification of proteins via the formation of ε-(γ-glutamyl)-lysine bonds between substrate proteins or through incorporation of many primary amines at the level of peptide-bound glutamine residues.[6] Tissue TG (tTG) has been revealed as the key autoantigen of the anti-endomysium antibodies (IgA type), which are diagnostic for celiac disease.[7] It has been hypothesized that the production of IgA anti-tTG antibodies is dependent on the help provided by gliadin-specific T cells to normally silent B cells specific for TG.[8] IgG antibodies against tTG (IgG anti-tTG Ab) can be found in other autoimmune diseases in humans and animals.[6,9] Generally, the cross-linking activity of tTG stabilizes apoptotic bodies and limits the leakage of intracellular components into the extracellular space. In

contrast, a reduction of this cross-linking is associated with the production of autoantibodies against tTG and an abnormal accumulation of LDH and double-stranded (ds) DNA in the blood.[6,10] Hence, a controlled disposal of cross-linked apoptotic bodies is required to prevent an inflammatory response as well as the exposure of self-antigens, which might lead to the development of autoimmunity.[11-13] TG is up-regulated upon receiving stress or cellular insult and often the enzyme is externalized or leaks into the matrix.[14] This alteration of the delicate balance between cell survival and death has been implicated in the pathogenesis of many autoimmune diseases.[15-17] The major disruptions in cellular homeostatic mechanisms has resulted in these enzymes contributing to a vast number of human diseases such as autoimmune diseases, neurodegenerative diseases, infectious diseases (hepatitis C and AIDS), inflammatory diseases including wound healing, tissue repair and fibrosis.[18-19]

The aim of this study was to find out the prevalence of anti-tTG antibodies in patients with AITD – Hashimoto's thyroiditis and Graves' disease. Likewise, it was of interest to ascertain whether the anti-tTG positivity has any relationship to thyroid function or thyroid antibodies. There are also abundant reports supporting the relationship between autoimmune disease and unregulated apoptosis, yet the mechanisms remain hypothetical.[20-21] Thus, TG involvement in the pathogeneses of common autoimmune diseases requires further investigation and clarification of the anti-tTG autoantibodies may be of great interest in relation to the diagnosis and follow-up of autoimmune diseases and may throw a new light on their etiology. Therefore, we propose that anti-tTG antibodies will be more prevalent in patients with thyroid autoantibodies, a marker used in the evaluation of AITD.

II. MATERIALS AND METHODS

1. Subjects

We examined a total of 155 subjects between the ages of 19 and 81 years; the average age was 42.9 years. The patient group consisted of 127 adults with thyroid disorders (102 women and 25 men; age, mean ± standard deviation (SD), 42.8 ± 13.4 years). The normal group included 28 healthy individuals matched for gender and age (22 women and 6 men, 43.5 ± 12.6 years). None of the patients had a previous diagnosis of an associated celiac disease. A retrospective chart review was conducted for all patients and normal group to assess whether these individuals had a previous diagnosis of celiac disease.

2. Methods

Sera were selected from our serum bank, in which they were stored at -70℃ for periods between 1 week to 5 years and more recently collected sera were stored at -20℃ for less than 1 week. The serum levels of T3 and free T4 (fT4) were measured using a competition electrochemiluminescence immunoassay (ECLIA) (Elecsys T3 Reagent and Elecsys FT4 Reagent, respectively; Roche, Mannheim, Germany) and serum TSH concentrations were assayed with a sandwich ECLIA method (Elecsys TSH Reagent, Roche, Mannheim, Germany; reference range: 0.86-4.69 μIU/mL). These thyroid function tests were analyzed on the Modular E-170 (Roche Diagnostics, Mannheim, Germany). The anti-TPO Ab and anti-thyroglobulin Ab were initially measured using semi-quantitative microtiter particle agglutination assays (Serodia-AMC, Fujirebio Inc, Tokyo, Japan). The more recent samples that were collected within 1

year were tested for anti-TPO Ab and anti-thyroglobulin Ab with a chemiluminescent sequential immunometric assay from Diagnostics Products Corporation (DPC, Los Angeles, CA, USA) using Immulite 2000 Analyzer (DPC, Los Angeles, CA, USA). The serum calcium levels (reference range: 8.7-10.8 mg/dL) were measured by the usual laboratory procedures and BMI (body mass index; reference interval: 18.5-24.9) was determined from retrospective chart review.

The sera of the patients and the healthy individuals were examined for the presence of IgA and IgG anti-tTG antibodies. IgA and IgG anti-tTG antibodies were assayed using a commercially available ELISA kit (Immuno-Biological Laboratories, Germany) which utilizes microplates coated with highly purified tTG. Measurements were carried out with two sets of the kit in the same laboratory and by a single operator. Sera were thawed only once before determinations. All patient and normal samples were diluted 1:100 with sample buffer according to the manufacturer's instructions. Controls, calibrators and prediluted patient and normal samples were pipetted into microwells containing human recombinant tTG. After a 30-minute incubation period at room temperature, the wells were washed to remove unbound serum antibodies. The horseradish peroxidase labeled enzyme conjugate solution containing polyclonal rabbit anti-human IgA was dispensed into the wells to allow the formation of a conjugate-antibody-antigen complex. The excessive enzyme conjugate which is not specifically bound was removed by washing the microwells after 15 minutes of incubation. The color of the solutions formed a blue color during the 15-minute incubation period on addition of a chromogenic substrate solution containing TMB (3,3',5,5'-Tetramethyl-benzidine). Color development was stopped by adding 1 M hydrochloric acid as a stop

solution. This reaction formed a yellow-end product; the intensity of this solution was measured photometrically at 450 nm. The amount of color was directly proportional to the concentration of IgA antibodies present in the original sample. The optical density for each calibrator was graphically plotted against the concentration of IgA and unknowns extrapolated from the curve. Testing for IgG anti-tTG was done with the same protocol except for the use of polyclonal rabbit anti-human IgG.

The reference range of the anti-tTG test given by the manufacturer was 10 U/mL. However, following recommendations for establishing normal and pathological ranges for each laboratory, we considered as positive a concentration of anti-tTG greater than 2 standard deviations above the mean obtained in the normal group. The cut-off was adjusted to 2.07 U/mL for IgA anti-tTG and 4.38 U/mL for IgG anti-tTG (these two cut-off limits were used for analysis throughout the study).

3. Statistical analysis

The results were statistically evaluated by t-tests, Mann-Whitney tests, Kruskal-Wallis tests, χ^2 tests or Fisher's exact tests with Analyse-it for Microsoft Excel (Analyse-It Software, Ltd. Leeds, UK) and all statistical analyses were graphed using GraphPad Prism 4 for Windows (GraphPad Software Inc., San Diego, California, USA). A probability value of $p<0.05$ was considered statistically significant.

III. RESULTS

1. Clinical characteristics and anti-tissue transglutaminase antibodies in normal and patient groups

The clinical features such as age, sex, BMI, serum calcium levels, DM, hypertension did not differ significantly between the normal and patient groups. The mean serum TSH values were significantly higher in the patient group than the normal group (mean ± SD; 9.59 ± 24.1 vs 2.03 ± 0.88 uIU/mL, p=0.0006). The proportion of IgA and IgG anti-tTG positive individuals did not significantly differ between the patient and normal groups as shown in Figure 1 (IgA: 19.7% vs 7.1%, p=0.1906, χ^2=1.71; IgG: 15.0% vs 7.1%, p=0.43, χ^2=0.62). In contrast, the median serum IgA and IgG anti-tTG Ab values were significantly higher in the patient group compared to the normal group (IgA: 1.882 vs 1.82 U/mL, p=0.0112; IgG: 3.442 vs 3.225 U/mL, p=0.0072, Figure 2).

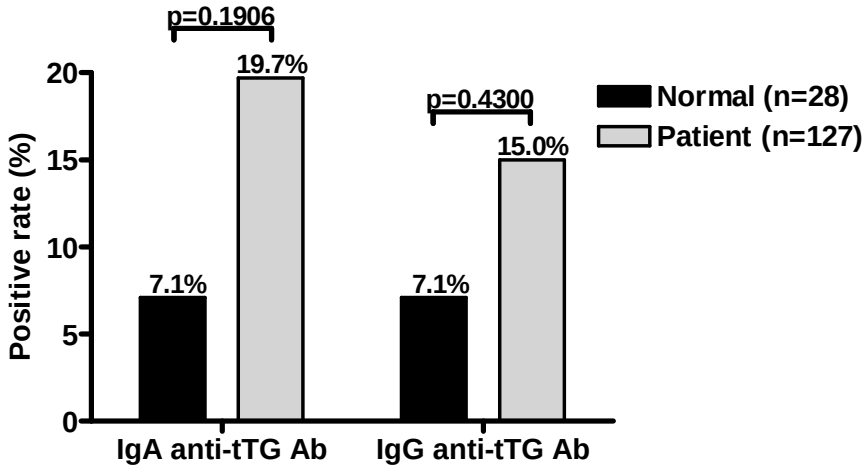


Fig. 1. The positive rate of IgA and IgG anti-tissue transglutaminase antibodies in normal and patient groups. Anti-tTG Ab: anti-tissue transglutaminase antibody, p=level of significance (χ^2 test).

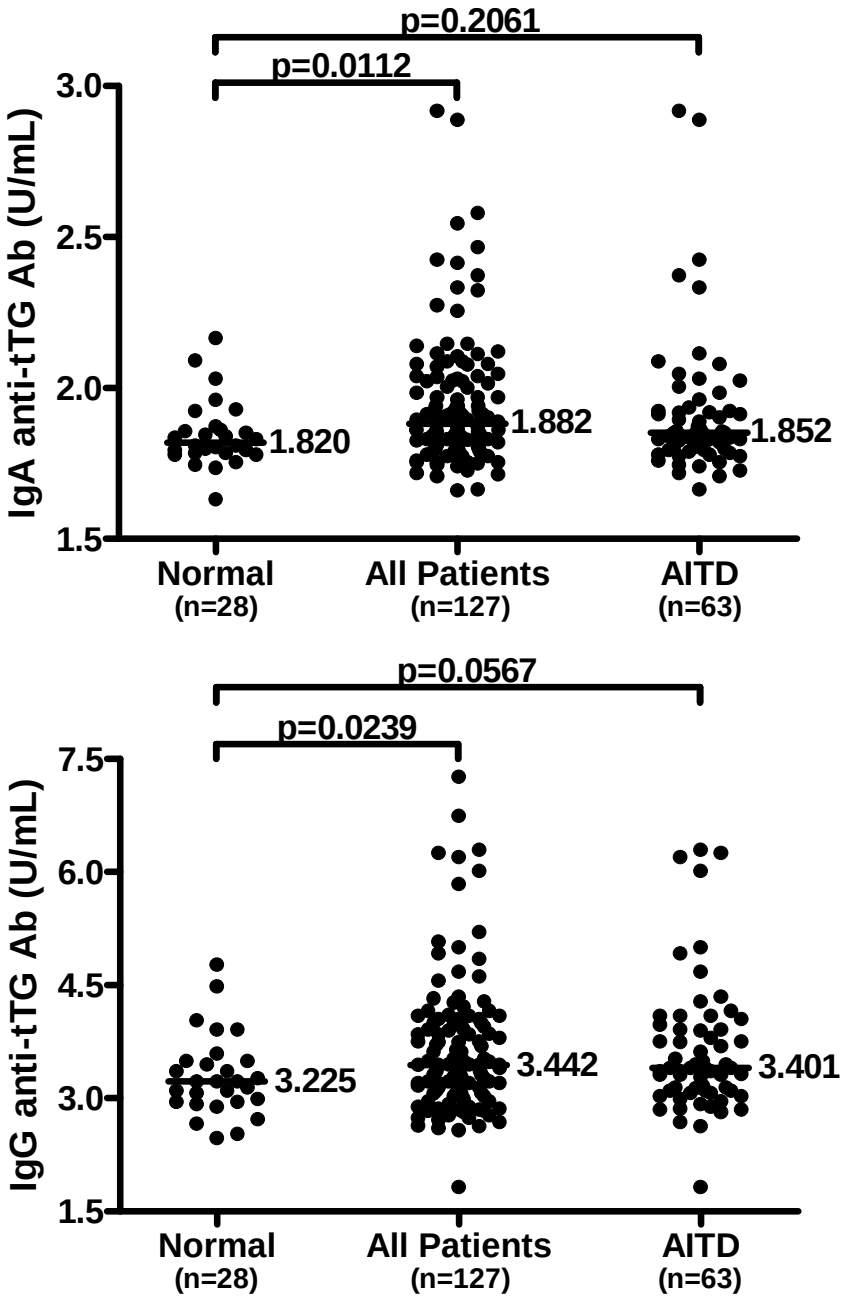


Fig. 2. Comparison of median anti-tissue transglutaminase antibody titers in the patient and AITD group compared to the normal group. Anti-tTG Ab: anti-tissue transglutaminase antibody, AITD: autoimmune thyroid disease, p=level of significance (Mann-Whitney test).

2. Clinical characteristics and anti-tissue transglutaminase antibodies in patients with autoimmune thyroid disease, thyroid cancer and other thyroid disorders

The AITD group consisted of 63 (35 HT + 28 GD) out of 127 subjects in the patient group. There were no significant differences between the AITD and normal groups in clinical characteristics such as age, sex, BMI, and serum calcium levels, and the occurrence of DM or hypertension as demonstrated in Table 1. The mean serum TSH level was significantly elevated in the AITD group (9.32 ± 24.16 vs 2.03 ± 0.88 μIU/mL, p=0.02, Table 1). Although there was no significant difference, the median values of IgA and IgG anti-tTG were slightly higher in the AITD group in comparison to the normal group (IgA: 1.852 vs 1.82 U/mL, p=0.2061; IgG: 3.401 vs 3.225 U/mL, p=0.0567) as illustrated in Figure 2. Neither the AITD nor the normal group showed any disparity in the positive rates of IgA and IgG

Table 1. Clinical characteristics of the normal and autoimmune thyroid disease (AITD) groups.

Parameter	Normal (n=28)	AITD (n=63)	p-value
Age (yr)	43.5 ± 12.6	42.2 ± 15.6	NS*
Sex (M / F)	6 / 22	9 / 54	NS†
BMI‡	23.3 ± 3.5	23.1 ± 2.5	NS*
Calcium (mg/dL)§	9.7 ± 0.4	9.7 ± 0.5	NS*
TSH (uIU/mL)‖	2.03 ± 0.88	9.32 ± 24.16	0.02*
Hypertension (n)‖	0	8	NS†
DM (n)‖	1	5	NS†

AITD: autoimmune thyroid disease, BMI: body mass index, TSH: thyroid stimulating hormone, DM: diabetes mellitus, NS: not significant.

The data for age, BMI, calcium, and TSH are give as mean ± standard deviation.

*T-test, †Fisher exact test, ‡40 missing values, §38 missing values, ‖2 missing values.

anti-tTG Ab (IgA and IgG: 12.7% vs 7.1%, p=0.6752, χ^2=0.18).

The AITD group was divided into two subgroups according to their affected disease, GD or HT. There were no significant differences in age, BMI, DM, and hypertension between the two groups. There was a significant decrease in mean serum calcium levels in HT patients compared to GD patients (9.3 ± 0.4 vs 10.0 ± 0.4, p=0.0006, Table 2). The mean serum TSH concentration in HT patients (14.81 ± 29.93 uIU/mL) was significantly higher than the normal group (p=0.0164) and GD patients (2.45 ± 11.14 uIU/mL, p=0.029, Table 2) whereas no significant difference was seen between the GD and normal group. There was a significant decrease in the mean serum T3 (116.1 ± 34.1 vs 338.0 ± 179.2 ng/dL, p<0.0001) and fT4 (1.1 ± 0.4 vs 4.2 ± 2.4 ng/dL, p<0.0001) levels in the HT group in comparison to the GD patients. There was no significant difference in the positive rates of anti-TPO Ab (91% vs 96%) and anti-thyroglobulin Ab (60% vs 71%) between HT and GD groups. Likewise, neither groups showed significant differences between the positive rates of IgA and IgG anti-tTG Ab (IgA and IgG: 14.3% vs 10.7%, p=0.9754, Table 2). The slight increase in median serum titers of IgA anti-tTG Ab in HT patients (1.855 U/mL) in comparison to the normal (1.820 U/mL, p=0.0927) or the GD group (1.833 U/mL, p=0.2396) was not of significance (Table 2). In addition, no significant difference in median IgA anti-tTG Ab was observed between the GD and normal groups. The median IgG anti-tTG Ab concentrations in HT patients were significantly higher than the normal group (3.527 vs 3.225 U/mL, p=0.0132) but not significantly higher than the GD group (3.240 U/mL, p=0.0667) as shown in Table 2. There was no significant

Table 2. Comparison of clinical characteristics and anti-tissue transglutaminase antibodies in patients with Graves' disease and Hashimoto's thyroiditis.

Parameter		Graves' disease (n=28)	Hashimoto's thyroiditis (n=35)	p-value
Age (yr)		38.2 ± 13.6	45.5 ± 16.5	NS*
Sex (M / F)		8 / 20	1 / 34	0.0098†
BMI§		23.3 ± 2.6	22.9 ± 2.5	NS*
Calcium (mg/dL)‖		10.0 ± 0.4	9.3 ± 0.4	0.0006*
TSH (uIU/mL)		2.45 ± 11.14	14.81 ± 29.93	0.0290*
Hypertension (n)¶		3	5	NS†
DM (n)¶		3	2	NS†
IgA anti-tTG Ab	n (positive)**	3 (10.7%)	5 (14.3%)	NS†
	median titer	1.833	1.855	NS‡
IgG anti-tTG Ab	n (positive)††	3 (10.7%)	5 (14.3%)	NS†
	median titer	3.24	3.527	NS‡

BMI: body mass index, TSH: thyroid stimulating hormone, DM: diabetes mellitus, anti-tTG Ab: anti-tissue transglutaminase antibody, NS: not significant.

The data for age, BMI, calcium, TSH are given as mean ± standard deviation.

*T-test, †Fisher exact test, ‡Mann-Whitney test.

§39 missing values, ‖36 missing values, ¶3 missing values.

**cut-off value for IgA anti-tTG Ab: 2.07 U/mL, ††cut-off value for IgG anti-tTG Ab: 4.38U/mL.

difference in median IgG anti-tTG Ab titers between GD and normal groups.

The thyroid cancer group was composed of 32 patients who had been previously diagnosed with thyroid cancer. No significant differences were seen between the thyroid cancer and normal groups in age, sex, BMI, serum calcium and TSH levels, and the occurrence of DM or hypertension. The thyroid cancer patients had significantly elevated median IgA anti-tTG Ab (1.955 vs 1.82 U/mL, p=0.0001) as well as IgG anti-tTG Ab (3.442 vs 3.225 U/mL, p=0.047) titers compared to the normal group. In addition, the median IgA anti-tTG Ab concentration in the thyroid cancer patients was significantly higher than the

AITD group (p=0.0017, Figure 3). However, the median titers of IgG anti-tTG Ab showed no significant difference between the thyroid cancer and AITD groups (p=0.6309, Figure 3). The positive rate of IgA anti-tTG Ab was significantly higher in the thyroid cancer group in comparison to the normal group (37.5% vs 7.1%, p=0.0136, χ^2=6.09) and the AITD group (37.5% vs 12.7%, p=0.0112, χ^2=6.43). In contrast, the IgG anti-tTG Ab did not show any statistical difference between the thyroid cancer and normal groups (18.8% vs 7.1%, p=0.3478, χ^2=0.88) as well as the thyroid cancer and AITD groups (18.8% vs 12.7%, p=0.6311, χ^2=0.23).

Thirty-two out of 127 patients had thyroid disorders such as thyroid mass or nodule, hypothyroidism, or hyperthyroidism without evidence of GD, HT, or thyroid cancer. There were no significant differences in age, sex, BMI, serum calcium levels, and the occurrence of DM or hypertension between the other and normal groups. The other group showed significantly increased mean serum TSH concentration compared to the normal group (11.93 ± 25.41 vs 2.03 ± 0.88 μIU/mL, p=0.0353). The median IgA anti-tTG Ab (1.882 vs 1.82 U/mL, p=0.0245) and IgG anti-tTG Ab (3.585 vs 3.225 U/mL, p=0.0372) titers were significantly higher compared to the normal group. However, the other group did not have significantly different median titers of IgA and IgG anti-tTG Ab compared to the AITD and thyroid cancer groups (Figure 3). No significant differences in IgA and IgG anti-tTG Ab positive rates were seen between the other and normal groups (IgA: 15.6% vs 7.1%, p=0.5366, χ^2=0.38; IgG: 15.6% vs 7.1%, p=0.5366, χ^2=0.38). Likewise, the

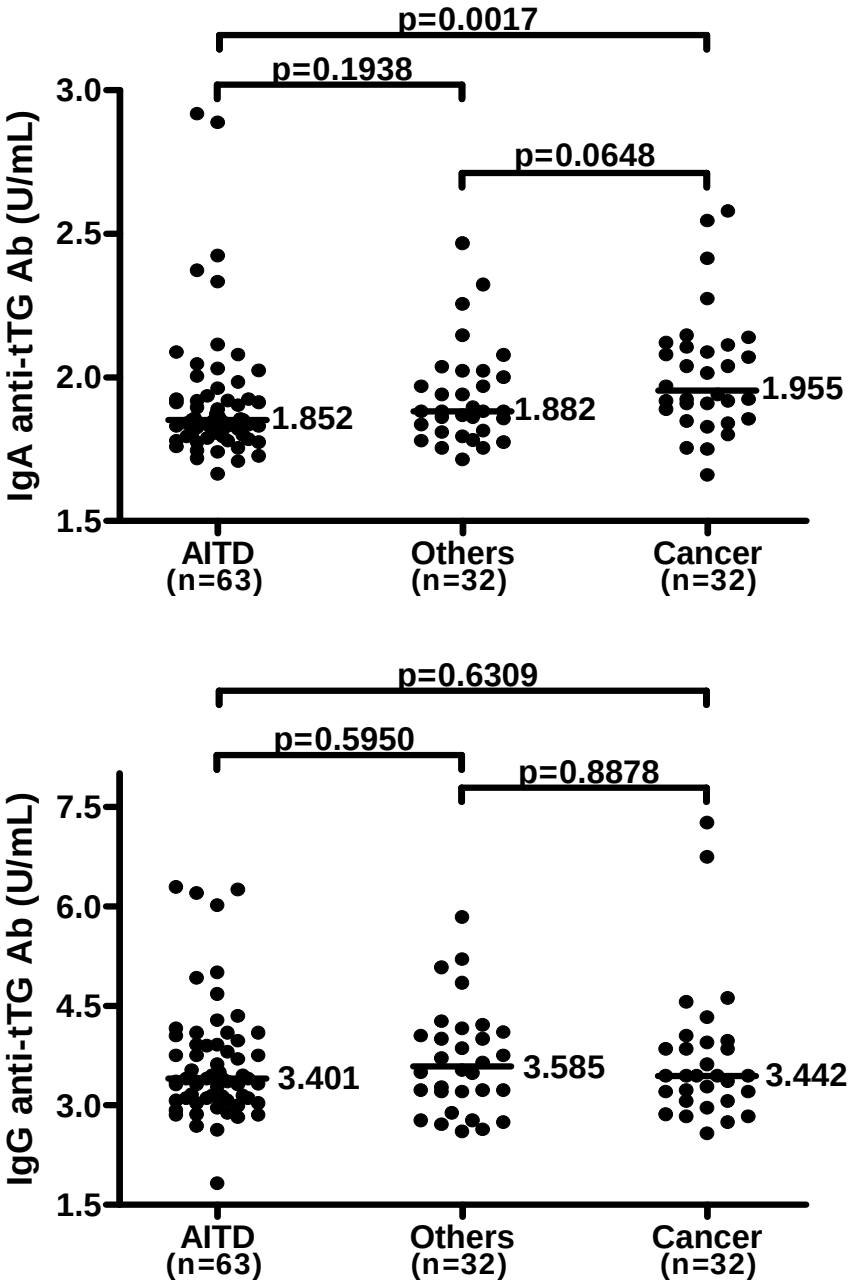


Fig. 3. Comparison of median anti-tissue transglutaminase antibody titers between the AITD, other, and thyroid cancer groups. Anti-tTG Ab: anti-tissue transglutaminase antibody, AITD: autoimmune thyroid disease, p=level of significance (Mann-Whitney test).

positive rates of IgA and IgG anti-tTG Ab in the other group did not differ significantly compared to the AITD or thyroid cancer groups.

3. Comparison of clinical characteristics between anti-tissue transglutaminase antibody positive and negative groups

Twenty-seven individuals out of 155 showed IgA anti-tTG positivity, including all patients and healthy individuals. The frequency of DM or hypertension did not differ significantly according to IgA anti-tTG Ab positivity. The mean age, BMI, serum calcium, T3, fT4 levels did not show any significant differences. The mean serum concentrations of TSH was significantly higher in the IgA anti-tTG Ab negative group (9.68 ± 24.1 vs 1.9 ± 3.2 uIU/mL, p=0.0007). The positive rate of anti-TPO Ab (52% vs 72.5%) and anti-thyroglobulin Ab (44% vs 52.9%) were not significantly different between the IgA anti-tTG Ab positive and negative groups.

IgG anti-tTG positive results were observed in 21 out of 155 individuals, including the normal group in this study. There was no significant difference between the IgG anti-tTG Ab positive and negative groups for DM and hypertension prevalence, mean age, BMI, serum calcium and TSH levels. Serum levels of T3 (194.3 ± 149.3 vs 118.6 ± 80.2 ng/dL, p=0.011) and fT4 (2.0 ± 1.8 vs 1.4 ± 0.9 ng/dL, p=0.0187) were significantly increased in the IgG anti-tTG Ab negative group. There was no significant difference in the positive rate of anti-TPO Ab (73.7% vs 67.6%) and anti-thyroglobulin Ab (47.4% vs 51.9%) between the IgG anti-tTG Ab positive and negative groups.

4. Association between anti-tissue transglutaminase and anti-thyroid antibodies

A total of 87 patients out of 127 were positive for anti-TPO antibodies. The mean age, BMI, serum TSH and calcium levels as well as the incidence of DM or hypertension did not differ significantly between the anti-TPO Ab positive and negative groups. The mean serum T3 (195.6 ± 151.7 vs 116.3 ± 49.5 ng/dL, p=0.0005) and fT4 levels (2.2 ± 2.0 vs 1.4 ± 0.7 ng/dL, p=0.002) were significantly higher in the anti-TPO Ab positive group. The positive rates of both IgA and IgG anti-tTG antibodies (IgA: 14.9% vs 30%; IgG: 16.1% vs 12.5%) showed no significant difference between the anti-TPO Ab positive and negative groups. Similarly, neither group diverged significantly in their medians of serum concentrations of IgG anti-tTG (3.442 vs 3.442 U/mL). On the contrary, the median serum IgA anti-tTG levels were significantly elevated in the anti-TPO Ab negative group (1.94 vs 1.855 u/mL, p=0.0008).

As illustrated in table 3, there was no significant difference in IgA and IgG anti-tTG Ab positive rates in association with

Table 3. Association between anti-tissue transglutaminase and anti-thyroid antibodies in patients with autoimmune thyroid disease (n=63).

		IgA anti-tTG Ab			IgG anti-tTG Ab		
		pos	neg	p-value*	pos	neg	p-value*
Anti-thyroid antibody		n (%)	n (%)		n (%)	n (%)	
Anti-TPO Ab	pos	7 (11.1)	52 (82.5)	0.8549	8 (12.7)	51 (81.0)	1.0000
	neg	1 (1.6)	3 (4.8)		0 (0.0)	4 (6.3)	
Anti-Thyroglobulin Ab	pos	5 (7.9)	36 (57.1)	1.0000	4 (6.3)	37 (58.7)	0.5629
	neg	3 (4.8)	19 (30.2)		4 (6.3)	18 (28.6)	

Anti-tTG Ab: anti-tissue transglutaminase antibody, anti-TPO Ab: anti-thyroid peroxidase antibody, pos: positive, neg: negative.
*Fisher exact test.

anti-TPO Ab in AITD patients. Likewise, the IgA and IgG anti-tTG Ab proportions did not differ significantly among anti-TPO positive and negative patients in GD, HT, thyroid cancer, and other groups (Table 4).

Thyroglobulin antibodies were positive in 65 out of 127 patients. The mean serum TSH, T3, fT4, and calcium levels as well as the mean age and BMI, the prevalence of DM or hypertension did not show any significant difference between the anti-thyroglobulin positive and negative groups. There were no significant differences in the positive rates (IgA: 16.9% vs 22.6%; IgG: 13.8% vs 16.1%) as well as the median serum titers (IgA: 1.862 vs 1.89 U/mL; IgG: 3.442 vs 3.442 U/mL) of both IgA and IgG anti-tTG antibodies between the anti-thyroglobulin Ab positive and negative groups.

Table 4. Association between anti-tissue transglutaminase and anti-thyroid peroxidase (TPO) antibodies.

		IgA anti-tTG Ab			IgG anti-tTG Ab		
		pos	neg	p-value*	pos	neg	p-value*
Anti-TPO Ab		n (%)	n (%)		n (%)	n (%)	
GD	pos	3 (10.7)	24 (85.7)	1.0000	3 (10.7)	24 (85.7)	1.0000
	neg	0 (0.0)	1 (3.6)		0 (0.0)	1 (3.6)	
HT	pos	4 (11.4)	28 (80.0)	0.7594	5 (14.3)	27 (77.1)	1.0000
	neg	1 (2.9)	2 (5.7)		0 (0.0)	3 (8.6)	
CA	pos	1 (3.1)	7 (21.9)	0.2008	2 (6.3)	6 (18.8)	0.9525
	neg	11 (34.4)	13 (40.6)		4 (12.5)	20 (62.5)	
Other	pos	5 (15.6)	15 (46.9)	0.1540	4 (12.5)	16 (50.0)	0.4160
	neg	0 (0.0)	12 (37.5)		1 (3.1)	11 (34.4)	

GD: Graves' disease, HT: Hashimoto's thyroiditis, CA: thyroid cancer, anti-tTG Ab: anti-tissue transglutaminase antibody, TPO:anti-thyroid peroxidase antibody, pos: positive, neg: negative.

*Fisher exact test

The IgA and IgG anti-tTG Ab positive rates did not differ significantly among anti-thyroglobulin positive and negative patients in the AITD group (Table 3). Likewise, there was no significant difference in IgA and IgG anti-tTG Ab proportions in association with anti-thyroglobulin Ab in GD, HT, thyroid cancer, and other groups (Table 5).

IV. DISCUSSION

There have been several studies with conflicting results of the association between CD and autoimmune disease such as AITD and SLE. Although anti-tTG antibodies are currently used in the diagnostic work-up of CD, there have been reports demonstrating the presence of anti-tTG

Table 5. Association between anti-tissue transglutaminase and anti-thyroglobulin (Tg) antibodies.

		IgA anti-tTG Ab			IgG anti-tTG Ab		
		pos	neg	p-value*	pos	neg	p-value*
Anti-Tg Ab		n (%)	n (%)		n (%)	n (%)	
GD	pos	2 (7.1)	18 (64.3)	1.0000	2 (7.1)	18 (64.3)	1.0000
	neg	1 (3.6)	7 (25.0)		1 (3.6)	7 (25.0)	
HT	pos	3 (8.6)	18 (51.4)	1.0000	2 (5.7)	19 (54.3)	0.6128
	neg	2 (5.7)	12 (34.3)		3 (8.6)	11 (31.4)	
CA	pos	3 (9.4)	4 (12.5)	1.0000	1 (3.1)	6 (18.8)	1.0000
	neg	9 (28.1)	16 (50.0)		5 (15.6)	20 (62.5)	
Other	pos	3 (9.4)	14 (43.8)	1.0000	4 (12.5)	13 (40.6)	0.4160
	neg	2 (6.3)	13 (40.6)		1 (3.1)	14 (43.8)	

GD: Graves' disease, HT: Hashimoto's thyroiditis, CA: thyroid cancer, anti-tTG Ab: anti-tissue transglutaminase antibody, Tg:anti-thyroglobulin antibody, pos: positive, neg: negative.

*Fisher exact test

in other autoimmune or inflammatory disorders. In a previous study on antinuclear antibodies, high concentrations of IgG anti-tTG were found in patients with SLE which suggests there is a link between apoptosis and autoimmunity.[22] Likewise, according to recent studies, 11% of samples from rheumatoid arthritis patients and 6.1% of samples from rheumatoid factor positive patients were positive for anti-tTG antibodies.[23-24] There is inconsistent information about the prevalence of anti-tTG antibodies in patients with AITD throughout the published reports; they have been reported to be as low as 2.7% and up to 14.8% in a more recent study.[3-4] In our study, we found increased anti-tTG antibodies in 20% of patients with various thyroid disorders. A positive rate of 12.7% was identified in the 68 AITD patients examined in our study for both IgA and IgG anti-tTG antibodies. The median titers for the transglutaminase antibodies were elevated (although not statistically significant in IgA titers) in HT patients in comparison to GD and normal groups, which is in agreement with an earlier report indicating a higher prevalence and higher levels of tTG antibodies in HT patients compared to GD patients.[2] Moreover, patients with HT were reported to have a higher risk of developing CD than patients with GD and correspondingly, CD patients seemed to develop hypothyroidism rather than GD.[2]

The frequency of celiac disease in patients with autoimmune thyroid disease has been reported to be 3-5%, which is much higher than the prevalence of celiac disease in the general population (0.3%).[3,25-26] Hence, it is important to consider the diagnosis of CD because it is a disease with a relatively high prevalence but easily overlooked with reports pointing out that up to 80% remain undiagnosed, causing latent clinical problems. Early identification of celiac disease in these patients will help prevent complications of untreated celiac disease such as ulcerative jejuno-ileitis, intestinal lymphoma, and other neoplasms and an

improvement of the associated autoimmune disease may be anticipated as well.[3,27] In spite of this, evaluation for CD such as an intestinal biopsy may not be considered until a patient complains of clinical symptoms highly suggestive of CD because CD is not known to be as prevalent in the Korean population. Even then, since gastrointestinal symptoms are nonspecific as well as being common in both autoimmune thyroid disorders and CD, it may be difficult to differentiate between the disease entities without a confirmatory procedure. Consequently, a screening test for CD may be carried out with anti-tTG antibodies which require a simple venipuncture to obtain sera from patients with AITD. As lower titers of these antibodies may not be disease specific, only high concentrations should be strongly associated with CD, and thus can avoid excessive invasive procedures such as intestinal biopsies with the inclusion of this "screening" process. Accordingly, a confirmatory intestinal biopsy for CD should be performed in the two patients with high titers of IgG anti-tTG in our study. A positive biopsy result will indicate an overt disease in cases with clinical symptoms and a subclinical disease without clinical symptoms. Patients with positive antibodies but without histopathological mucosal changes and clinical features may be designated as silent disease CD cases, which may warrant careful follow-up for progression into the subclinical or overt celiac disease.

TPO and thyroglobulin antibodies are seen in nearly all patients with HT and approximately 50-70% of GD patients have a combination of these antibodies. In our study, TPO antibodies were positive in over 90% of the patients in both HT and GD patients and curiously, the anti-thyroglobulin antibodies showed higher prevalence in GD patients. This may be due to the fact that patients with long-standing thyroid diseases undergoing treatment and/or follow-up had been included in the study. A

reduction in thyroglobulin antibody titers may have occurred in some of these patients with favorable response to treatment. In addition, the presence of these antibodies was noted in patients with other thyroid disorders. Twenty out of 32 patients designated to the other group were positive for anti-TPO antibodies and 12 of them were concurrently positive for anti-thyroglobulin antibodies. Moreover, 12 of the TPO-positive patients had hypothyroidism, which may indicate a state of thyroid autoimmunity, without progression to autoimmune thyroid disease. Another finding that may be of interest is the high prevalence of IgA anti-tTG antibodies in TPO-negative patients. Of the 40 anti-TPO negative patients, 24 were patients were diagnosed with thyroid cancer and 12 were categorized into the other thyroid disorder group. Twenty out of 24 TPO-negative thyroid cancer patients were simultaneously negative for thyroglobulin antibodies; 7 were positive for IgA anti-tTG antibodies, 3 were IgG anti-tTG-positive and 1 patient showed positive results for both IgA and IgA anti-tTG antibodies. The IgA and IgG anti-tTG positive patient, who had the highest IgG titer among the 155 tested individuals, had undergone surgery for a thyroidectomy approximately 4 months ago and has been taking Levothyroxine since then for thyroid hormone replacement. Given that the tTG antigen is expressed in many organs and it its known to be released upon tissue damage, we may be able to assume that a nonspecific increase may be seen in thyroid cancer patients, possibly due to post-operative inflammatory states.[28] However, a thyroid cancer patient scheduled to receive surgical treatment showed TPO-negative and IgA ant-tTG positive results, indicating that the transglutaminase antibodies may not be an incidental finding due to post-operative changes. Thus, it may be interesting to further evaluate thyroid cancer patients before and after surgery to evaluate for changes in prevalence or titers of anti-thyroglobulin antibodies.

The somewhat arbitrary designation of the cutoff level at 2.07 and 4.38 U/mL for IgA and IgG anti-tTG antibodies, respectively may have caused false positive results in some of the cases especially since only a relatively small number of healthy individuals used to determine this level. Nevertheless, the mean ± 2SD values was comparable to the 95% upper limit value of the reference interval calculated by the parametric method. The four healthy subjects who were positive for either IgA or IgG anti-tTG antibodies had titers that would have been considered negative upon employing a mean ± 3SD limit for the cut-off level. Hence, although our normal group consisted of healthy volunteers without any history of thyroid disorders, it might be recommended that a larger population for the normal group be used to establish the reference interval. Some other limitations to this study are that there may have been some inconsistency in the results because the study was based on a retrospective review of patients' records, and patients weren't always followed and monitored in the same manner. The lack of confirmatory intestinal biopsies is another limitation of this study, since celiac disease often goes undiagnosed and the clinical manifestations may be atypical.

In conclusion, although no association was seen between the anti-tTG antibodies and the presence of specific AITDs, the presence of these anti-tTG antibodies in patients with thyroid cancer and various other thyroid disorders suggests further investigation for clinical implications of autoantibodies in patients without known autoimmune disorders.

V. CONCLUSION

Tissue transglutaminase has been discovered to be a diagnostic marker for celiac disease and the IgG antibodies against tTG have been

reported to be found in other autoimmune diseases. Celiac disease has been closely associated with autoimmune thyroid disease, with roughly a 10-fold higher risk than the general population. Approximately 24% of the AITD patients examined in our study were positive for either IgA or IgG anti-tTG antibodies. One patient was positive for both IgA and IgG antibodies and 7 patients were IgA-positive and the remaining 7 were IgG-positive. However, no connection could be found between the anti-tTG antibodies and the presence of specific AITDs. There was no significant difference in positive rates of anti-tTG antibodies in association with anti-TPO and anti-thyroglobulin antibodies among GD, HT, thyroid cancer, and other thyroid disease groups. Yet, anti-tTG antibodies were observed in patients with thyroid cancer and other thyroid disorders such as hypothyroidism, which requires further investigation for clarification of the clinical implications of the transglutaminase antibodies in non-autoimmune disorders.

VI. REFERENCES

1. Collin P, Salmi J, Hallstrom O, Reunala T, Pasternack A. Autoimmune thyroid disorders and coeliac disease. Eur J Endocrinol 1994;130:137-140.
2. Valentino R, Savastano S, Tommaselli AP, Dorato M, Scarpitta MT, Gigante M, et al. Prevalence of celiac disease in patients with thyroid autoimmunity. Horm Res 1999;51:124-127.
3. Volta U, Ravaglia G, Grantio A, Forti P, Maioli F, Petrolini N, et al. Coeliac disease in patients with autoimmune thyroiditis. Digestion 2001:64:61-65.
4. Jiskra J, Limanova Z, Vanickova Z, Kocna P. IgA and AgG antigliadin, IgA anti-tissue transglutaimnase and antiendomysial antibodies in patients with autoimmune thyroid diseases and their relationship to thyroidal replacement therapy. Physiol Res 2003;52:79-88.
5. Vitoria JC, Arrieta A, Arranz C, Ayesta A, Sogo A, Maruri N, et al. Antibodies to gliadin, endomysium and tissue transglutaminase for the diagnosis of celiac disease. J Pediatr Gastroenterol Nutr 1999;29:571-574.
6. Piredda L, Amendola A, Colizzi V, Davies PJA, Farrace MG, Fraziano M, et al. Lack of ‘tissue’ transglutaminase protein cross-linking leads to leakage of macromolecules from dying cells: relationship to development of autoimmunity in MRLIpr/Ipr mice. Cell Death Differ 1997;4:463-472.
7. Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997;3:797-801.
8. Sollid LM, Molberg O, McAdam S, Lundin KE. Autoantibodies

in celiac disease: tissue transglutaminase-guilt by association? Gut 1997;41:851-852.

9. Lampasona V, Bonfanti R, Bazzigaluppi E, Venerando A, Chiumello G, Bosi G, et al. Antibodies to tissue transglutaminase C in type I diabetes. Diabetologia 1999;42:1195-1198.
10. Piacentini M, Colizzi V. Tissue transglutaminase: apoptosis versus autoimmunity. Immunol Today 1999;20:130-134.
11. Knight C, Hand D, Piacentini M, Griffin M. Characterization of the transglutaminase-mediated large molecular weight polymer from rat liver; its relationship to apoptosis. Eur J Cell Biol 1993;60:210-217.
12. Chervonsky AV. Apoptotic and effector pathways in autoimmunity. Curr Opin Immunol 1999;11:684-688.
13. Andrade F, Casciola-Rosen L, Rosen A. Apoptotis in systemic lupus erythematosus. Rheum Dis Clin North Am 2000;26:215-227.
14. Beninati S and Piacentini M. The transglutaminase family: an overview: minireview article. Amino Acids 2004;26:364-372.
15. Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature 1992;356:314-317.
16. Benoist C and Mathis D. Cell death mediators in autoimmune diabetes – no shortage of suspects. Cell 1997;89:1-3.
17. Giordano C, Stassi G, de Maria R, Todaro M, Richiusa P, Papoff G, et al. Potential involvement of Fas and its ligand in the pathogenesis of hashimoto's thyroiditis. Science 1997;275:960-963.
18. Kim SY, Jeitner TM, Steinert PM. Transglutaminases in disease.

Neurochem Int 2002;40:85-103.

19. Di Giovanna JJ, Robinson-Bostom L. Ichthyosis: etiology, diagnosis, and management. Am J Clin Dermatol 2003;4:81-95.
20. Ravirajan CT, Pittoni V, Isenberg DA. Apoptosis in human autoimmune diseases. Int Rev Immunol 1999;18:563-589.
21. Clemens MJ, Van Venrooij WJ, Van de Putte LBA. Apoptosis and autoimmunity. Cell Death Differ 2000;7:131-133.
22. Van der Sluijs Veer G, Vermes I. IgG autoantibodies against tissue transglutaminase in relation to antinuclear antibodies. Clin Chem 2001;47:952-954.
23. Feighery L, Collins C, Feighery C, Mahmud N, Coughlan G, Willoughby R, et al. Anti-transglutaminase antibodies and the serological diagnosis of celiac disease. Br J Biomed Sci 2003;60:14-18.
24. Song KS and Choi JR. Tissue transglutaminase autoantibodies in patients with IgM rheumatoid factors. Yonsei Med J 2004;45:960-962.
25. Maki M and Collin P. Coeliac disease. Lancet 1997;349:1755-1759.
26. Berti I, Trevisiol C, Tommasini A, Citta A, Neri E, Geatti O, et al. Usefulness of screening program for celiac disease in autoimmune thyroiditis. Dig Dis Sci 2000;45:403-406.
27. Marsh MN. Is celiac disease (gluten sensitivity) a premalignant disorder? J Pediatr Gastroenterol Nutr 1997;24:525-527.
28. Abdulkarim AS and Murray JA. Review article: the diagnosis of celiac disease. Aliment Pharmacol Ther 2003;17:987-995.

ABSTRACT (In Korean)

자가면역성 갑상선질환에서 항-조직 transglutaminase 항체 검색

<지도교수 송경순>

연세대학교 대학원 의학과

박서진

서론: IgA 항-조직 transglutaminase 항체 검사로 셀리악병(celiac disease) 진단이 가능하며 IgG 항-조직 transglutaminase 항체는 여러가지 자가면역성 질환에서 양성 소견을 보인다는 연구가 많이 있다. 셀리악병과 자가면역성 갑상선질환은 공통적으로 자가면역성을 병리기전으로 가지고 있으며 연관성을 알아보고자 자가면역성 갑상선질환 환자들에서 항-조직 transglutaminase 항체 검사를 시행하였다.

방법: 그레이브스병, 하시모토 갑상선염, 갑상선항진증, 갑상선저하증, 갑상선 결절, 갑상선암 등과 같이 갑상선 질환을 앓고 있는 환자 127명과 건강검진을 위해 내원한 건강한 성인 28명에서 항-조직 transglutaminase 항체 검사를 시행하였다.

결과: 갑상선 질환이 있는 환자들에서 IgA (1.882 U/mL)와 IgG (3.442 U/mL) 항-조직 transglutaminase 항체의 정중값이 정상 성인(IgA: 1.820 U/mL, IgG: 3.225 U/mL)보다 유의하게 높은 것으로 나타났다 (각각 p=0.0112와 p=0.0239). 그러나 항-조직 transglutaminase 항체의 양성율은 갑상선질환군 (IgA: 19.7%, IgG: 15.0%)과 정상 성인군 (IgA와 IgG: 7.1%)간에 통계적으로 유의한 차이를 보이지 않았다. 자가면역성 갑상선질환군 63명 중

8명은 (그레이브스병 3명 + 하시모토병 5명) IgA 항-조직 transglutaminase 항체에 양성소견을 보다. 또한 63명의 자가면역성 갑상선질환군 중에서 IgA 음성이었던 7명과 (그레이브스병 3명 + 하시모토병 4명) IgA 양성이었던 하시모토병 1명이 IgG 항-조직 transglutaminase 항체 양성이었다. 그러나 항-조직 transglutaminase의 양성율은 자가면역성 갑상선질환군과 정상 성인군 간에 차이를 보이지 않았다 (IgA와 IgG: 12.7% vs 7.1%). 갑상선 질환을 앓고 있는 환자군별로 혈청 갑상선항체와 항-조직 transglutaminase 항체의 양성율 간에 연관성은 보이지 않았다.

결론: 자가면역성 갑상선질환과 항-조직 transglutaminase 항체 간에는 연관성이 없는 것으로 나타났다. 그러나 갑상선암이나 다른 갑상선 질환 환자들이 보였던 더 높은 역가의 항-조직 transglutaminase 항체의 임상적 의의에 대한 연구가 더 필요할 것으로 사료된다.

핵심되는 말: 항-조직 transglutaminase 항체, 자가면역성 갑상선질환, 항-갑상선 항체